Characterization of Essential Oil from Malaysian Curry Leaves

JAMIL BIN ROSLAN

Bachelor of Chemical Engineering

Faculty of Chemical & Natural Resources Engineering

UNIVERSITI MALAYSIA PAHANG

JANUARY 2014

ABSTRACT

The objective of this research is to characterize the component in essential oils from M. koenigii leaves by hydro distillation extraction method. The major constituent of M. koenigii has been reported as caryophyllene and 3-carene which is responsible for the aroma and flavor. This research has focused on the chemical constituent of M.koenigii essential oil that grow in Malaysia. In this research, the methods of grinding, extraction, separation and analysis are used and the sample is separated from water by using a chemical with different polarity to get the essential oil. The sample was analyzed by using a GC-MS to identify the component of M.koenigii essential oil. In this research, the most optimum time of extraction also determine by using different length of time with correspond to the yield of essential oil. The major component in M. koenigii leaves is caryophyllene with the optimum time of extraction is 9 hour with the yield of essential oil is 0.22%. The compositions of essential oil also show the potential on biological and repellent activity.

ABSTRAK

Objektif kajian ini adalah untuk mencirikan komponen dalam minyak pati dari daun M. koenigii oleh kaedah pengekstrakan hidro penulenan. Konstituen utama M. koenigii telah dilaporkan sebagai caryophyllene dan 3-carene yang bertanggungjawab untuk aroma dan rasa. Kajian ini telah memberi tumpuan kepada konstituen kimia M.koenigii minyak pati yang tumbuh di Malaysia. Dalam kajian ini, kaedah pengisaran, pengekstrakan, pengasingan dan analisis digunakan dan sampel dipisahkan dari air dengan menggunakan bahan kimia dengan polariti yang berbeza untuk mendapatkan minyak pati. Sampel telah dianalisis dengan menggunakan GC-MS untuk mengenal pasti komponen M.koenigii minyak pati. Dalam kajian ini, masa yang paling optimum pengekstrakan juga ditentukan dengan menggunakan panjang masa yang berbeza dengan hasil minyak. Komponen utama dalam M. daun koenigii adalah caryophyllene dengan masa yang optimum pengekstrakan ialah 9 jam dengan hasil minyak adalah 0.22%. Komposisi minyak pati juga menunjukkan potensi aktiviti biologi dan penghalau serangga.

TABLE OF CONTENTS

Chapter	Title	Page
	DECLARATION	**ii**
	DEDICATION	**iii**
	ACKNOWLEDGEMENT	**iv**
	ABSTRACT	**v**
	ABSTRAK	**vi**
	TABLE OF CONTENT	**vii**
	LIST OF TABLES	**x**
	LIST OF FIGURES	**xi**
	LIST OF ABBREVIATIONS	**xiii**
	LIST OF APPENDICES	**xiv**
1	**INTRODUCTION**	
	1.1 Background of study	1
	1.2 Problem Statement	2
	1.3 Objective	3
	1.4 Scope of Research Work	3
	1.5 Organisation of this thesis	4
2	**LITERATURE REVIEW**	
	2.1 MURRAYA KOINIGII (L.) Spreng	5
	2.1.1 Chemical structure of M.koenigii leaves	6
	2.1.2 Uses of M.koenigii leave	8
	2.2 Essential Oil	9
	2.2.1 Chemical constituent of Essential Oil	10

2.3 Insect repellent 17
2.3.1 Repellent activity of essential oil 18
2.4 Extraction of essential oil 19

3 METHODOLOGY
3.1 Material 22
3.2 Pre-treatment Method 22
3.2.1 Weighing 22
3.2.2 Grinding 23
3.3 Hydro distillation extraction method 23
3.4 Separation method 23
3.5 Analysis method 24
3.5.1 Gas chromatography- mass spectrometer (GC-MS) analysis 24

4 RESULTS & DISCUSSIONS
4.1 Introduction 25
4.2 Yield essential oil of curry leaf 26
4.3 Hydro distillation extraction 26
4.3.1 Effect of different time of extraction 27
4.4 Characterization of curry leaf essential oil 30
4.4.1 Biological activity of essential oil 34
4.4.2 Potential of repellent activity 35

5 CONCLUSION & RECOMMENDATION
5.1 Conclusion 36
5.2 Recommendation 37

REFERENCES 38
APPENDIX A-I 43-96

LIST OF TABLES

Table No	Title	Page
2.1	Components of the essential oil of M.koenigii; only those substance are included that make up more than 0.2% of the oil and have been unequivocally	6
2.2	Characteristic of curry leaves essential oil	7-8
4.1	Chemical constituents, retention times and percentage area of oxygenated compound of curry leaf essential oil.	26
4.2	Comparison the yield percent of the essential oil	28
4.3	Functional group of the major component	30
4.4	Chemical constituents, retention times and percentage area of oxygenated compound of curry leaf essential oil.	31-32
4.5	Biological activity of the composition of essential oil	35

LIST OF FIGURES

Figure No	Title	Page
2.1	M.koenigii leaves	5
2.2	Isoprene	11
2.3	Chemical constituent in essential oil	15-16
2.4	Structure of several insect repellent	18
4.1	Effect of the extraction time on the yield of the curry leaf essential oil	27
4.2	Caryophyllene chemical structure	31

LIST OF ABBREVIATIONS

GC	-	Gas Chromatograph
MS	-	Mass Spectrometer
FKKSA	-	Fakulti Kejuruteraan Kimia & Sumber Asli
W	-	Watt
mN	-	Millinewton
cm	-	Centimeter
mmHg	-	Mlillimeter of Mercury
cP	-	Centipoise

LIST OF APPENDICES

Appendix	Title	Page
A	Gantt chart for Undergraduate Research Project 1 and 2	43
B	GC-MS analysis: Research 1	45
C	GC-MS analysis: Research 2	49
D	GC-MS analysis: Research 3	58
E	GC-MS analysis: Research 4	67
F	GC-MS analysis: Research 5	76
G	GC-MS analysis: Research 6	83
H	GC-MS analysis: Research 7	87
I	GC-MS analysis: Research 8	93

CHAPTER 1

INTRODUCTION

1.1 Background of study

Essential oils contain highly volatile substances that are isolated by a physical method or process from plants of a single botanical species. Essential oils are so termed as they are believed to represent the very essence of odor and flavor. Essential oil plants and culinary herbs include a broad range of plant species that are used for their aromatic value as flavorings in foods and beverages and as fragrances in pharmaceutical and industrial products. Some study also found that the essential oil have the properties against various haematophagous arthropods, and some of them being the basis of commercial repellent formulation.

M.koenigii or its common name curry leaf tree is the traditional spices used in south India for all curry preparation. The plant M.koenigii belonging to the family rutaceae is native to India and distributed in most part of southern Asia. Essential oil composition of leaves has been studied by various workers. The major constituent responsible for the aroma and flavor has been reported as pinene, sabinene, caryophyllene, cadinol, and cadinene (Anonymous, 1962; Nigam et al., 1961; Prakash et al., 1974; Macleod et al., 1982; Hiremath et al., 1998). Even though most of the study of the essential oil of curry leaf show the potential in biological activity, but none of them show the potential in the repellent activity.

There are several methods to extraction of essential oil from herb and spices but in this study will use common hydro distillation extraction to extract the oil from the leaves. This extraction method is most used in the industry to obtain the essential oil from the plant. The step required for the preparation of material prior to extraction the essential oil from the leaves and uses the Gas chromatography mass spectrometer (GC-MS) to analyse the composition of essential oil in detail. The analysis of the GC-MS will determine the active compound of the repellent activity in the essential oil.

1.2 Problem statement

The M.koenigii or curry leaves have been used as traditional medicine in eastern Asia as tonics for dysentery, fever, carminative and the bites of poisonous animal and sources of flavors. Many research also show the component of the essential oil such antioxidant, tocopherol, β-carotene and lutein (Palanishwamy,2001) have the pharmaceutical potential such as experimental for treatment of diabetes in rat (Arulselvan and Subramanian, 2006) and antibacterial activity against various human pathogenic bacteria (Ningappa et al., 2009). (citation)

The insect repellent has been introduced in World War 2 as for a formula for clothing in military. In that time, the repellent was develop using a combination of 3 synthetic chemical with formulation of 6-2-2; six parts dimethyl phthalate, two parts Indalone and two parts Rutgers (Peterson and Coats, 2001) but the it fall to provide desired protection of military personnel development around the world. In1956, the insect repellent properties of N, N-diethyl-m-toluamide (DEET) were discover and success to gives a protection from insect. But the problem of this the toxic effect from this synthetic chemical have been recorded, including encephalopathy in children, urticarial syndrome, anaphylaxis, hypotension and decreased heart rate (Peterson and Coats, 2001).

The previous research shows that mostly the constituent of the essential oil consist of the several compound that can be used as the repellent active compound. In this study is conducted in order to characterize the essential oil of the curry leaf using the hydro distillation extraction method and also use the advance equipment for the analysis. It also to find the present of the component inside the essential that can be used as the repellent for the insect.

1.3 Objectives

The objective of this research is to characterize the chemical composition present on the curry leaves essential oil.

1.4 Scope of this research

The following are the scope of this research:

- Preparation and extraction of M.koenigii leaves' essential oil
- Analysis of chemical composition of the essential oil using GC-MS
- Investigate the potential of repellent activity that may have present in essential oil
- Analysis the toxicity on the curry leaves.

1.5 Organisation of this thesis

There are 5 chapters in this thesis. Following the introduction, the remaining chapter in this thesis are organized as follows:

Chapter 2 begins with a description about the curry leaf as well as the composition and the uses of the curry leaves with the previous researches that related to the study. In this chapter also included a general description on the essential oil with a brief explanation about the chemical constituent present in essential oil. The repellent activity of essential oil also discussed in this chapter.

Chapter 3 starts with the methodology to extract the essential oil from the curry leaves. It involve with the pre-treatment on the preparation in the extraction process. After the essential oil successful obtained, it continued to the analysis using GC-MS. Analysis on toxicity of the curry leaves using AAS also covered in this chapter.

Chapter 4 commences with the analysis of the result and discussion

Chapter 5 concludes the overall findings in this work. Some recommendations for future work are presented here.

CHAPTER 2

LITERATURE REVIEW

2.1 MURRAYA KOINIGII (L.) Spreng

M.koenigii (L.) spreng is the aromatic small tree, that belonging to the citrus family, Rutaceae that grow widely in East Asia and in peninsular Malaysia, two species of *Murraya* was found. Originated in Tirai region of Uttar Pradesh, India, it is now widely found in parts of India and also culvative in Sri Lanka, China, Australia and Pacific Island. The plant was spread to Malaysia, South Africa and Reunion Island by south Asian immigrants. The *M.koenigii* plant has been widely used in traditional medicine and as a tonic to treat dysentery, fever, influenza stomachic, stimulants, carminative and bites of poisonous animal. The leaves of *M.koenigii* also may be medicinally useful for the treatment or prevention of diabetes, cancer, and possibly cardiovascular dieses (Dasguptaa, Raoa, & Yadava, 2003). The leaves of *M.koenigii* are widely use in Indian cookery for flavoring food stuff and also became spices after drying. Below is the figure of *M.koenigii* leaves:

Figure 2.1 *M.koenigii* leaves

It is believed that the folks in the rural areas of Malaysia use curry leaves as traditional home remedies for flies' prevention. In Malaysia, it is usual for the local folks to plant the tree at the back yard of their house which makes it more convenient for daily uses. The use of curry leaves, scientifically known as *M.koenigii* and called as daun kari in Malaysia is not new. The leaves have been widely applied in the culinary field due to its aromatic scents and natural flavoring especially in traditional cuisines such as curries and sauces.

2.1.1 Chemical composition of M.koenigii leaves

The curry leaves are rich in minerals, Vitamin A and B, proteins, amino acid and alkaloids (Kong et al., 1986; Tee & Lim, 1991). It also rich with calcium but the present of high concentration of oxalic acid. By the analysis of concentrated essence of *M.koneigii* from Macleod & Pieris (1982) and Quan li et al., (1988) study, they obtain that the component of the essential oil of *M.koenigii* as table below:

Component	Rel. amount [%]	
	Macleod & Pieris (1982)	Quan li et al., (1988)
α-Pinene	-	38.4
Camphene	-	0.5
Sabinene	-	0.3
β-Thujene	4.3	-
β-Pinene	0.7	6.3
α-phellandrene	0.6	-
Limonene	2.1	3.5
β-Phellandrene	6.1	0.5
trans-β-Ocimene	1.9	0.5
τ-Terpinene	-	0.3

α-Cubebene	0.2	-
α-Copaene	0.9	-
β-Elemene	6.8	0.8
β-Caryophyllene	28.7	12.9
Humulene	-	3.5
β-Cubebene	-	2.1
τ-Elemene	-	10.1
δ-Cadinene	-	1.1
β-Gurjunene	21.4	-
ε-Muurolene	0.4	-
β-Bisabolene	2.8	-
τ-Cadinene	2.5	-
α-Selinene	2.9	-

Table 2.1 Components of the essential oil of *M.koenigii*; only those substance are included that make up more than 0.2% of the oil and have been unequivocally

The composition of the essential oil *of M.koenigii* may be different at the different place. Like Sri Lanka, the composition of oil was reported to contain monoterpenes (15.9%) and sesquiterpenes(80.2%) with *β-phellandrene, β-caryophyllene, β-gurjunene, β-elemene*, and *α-selinene* as the main constituents whereas the composition oil from Chinese was reported contain α- and *β-pinenes*, *β-caryophyllene* and *γ-elemene* as main constituents. For the composition oil from Malaysia, it was shown to be rich in monoterpenes and oxygenated monoterpened (ca. 85%) with *α-pinene, limonene, β-phellandrene*, *terpinen-4-ol* and *β-caryophyllene* as the main contents (Bhattacherya et al., 1982). From Chowdhury study reported that the leaves on hydro distillation gave 0.5% essential oil on fresh weight basis, having dark yellow color, spicy odor and pungent clove- like taste. It has following characteristics:

Table 2.2 Characteristic of curry leaves essential oil

Specific gravity (25^0C)	00.9748
Refractive index (25^0C)	1.5021
Optical rotation (25^0C)	+ 4.8
Saponification value	5.2
Saponification value after acetylation	54.6
	66.3%
Moisture	6.1%
Protein	1.0%
Fat (ether extract)	6.4%
Fiber	4.2%
Mineral matter	801mg/100g of edible portion
Calcium	600 mg/100 g of edible portion
Phosphorus	3.1 mg/100 g of edible portion
Iron	126000 IU/100 g
Carotene (as vitamin A)	2.3 mg/100 g
Nicotinic acid	4 mg/ 100 g
Vitamin C	absent
Thiamine and riboflavin	

2.1.2 Uses of M.koenigii leaves

The different part of the *M.koenigii* plant has been use as a folk medicine in India. The leaves, bark and the root are used in indigenous medicine as tonic, stomachic, anthelmintic, analgesic and as stimulative, appetizing and carminative agent for treating piles, influenza, fever, itching, dropsy, bronchial asthma, eruptions and bites of poisonous animal, dysentery, diarrhea, body aches, fresh cuts, kidney pains and vomiting (Kumar, Sharma, Tiwari &Kumar, 1999; Rana,Juyal,,Rashmi&Blazquez,2004).

Ningappa et al., 2009 reported that the antioxidant protein (APC) that isolated from the *M.koenigii* leaves was exhibited a board spectrum of antibacterial activity against human pathogenic bacterial, compare to the commercial antibiotics in their study. The APC show that the antibacterial activity against Escherichia coli and Staphylococcus aureus. The results show that, the clear zone of inhibition (20-25 mm) was form by using 0.15µg of APC using agar diffusion method.

The other studies also show that the antioxidant properties inside the *M.koenigii* leaf are useful as anti-obesity for the high fatty diet rats (Sachin & Dinesh., 2012). There were lower of hydro peroxides, conjugatedienes and free fatty acid in the liver and hearth of the rats supplemented with *M.koenigii* leaves compared to rats fed on the high fat diet alone. As the *M.koenigii* leaves was supplemented, the activities of superoxide dismutase, catalase and glutathione transferase were increase in the hearth and liver of the rats as well as the increasing activities of glutathione reductase, glutathione peroxidase and glucose-6-phosphate dehydrogenase in the liver. At the same time the concentration of gluthathione was decrease in the liver. Thus the supplementing a high fat diet with 10% of *M.koenigii* leaf can prevent the formation of free radicals and maintain the tissue at normal level

2.2 Essential Oil

Essential oil is the concentrated liquid that generally steam or hydro-distilled from leaves, flowers, roots of plant and trees that containing the volatile aroma compound that can be represent the characteristic flavor to the plant part. These compound usually responsible the aroma and the flavor that associated with the herbs, spices and perfume. The formation and accumulation of essential oil in plant have been thoroughly review by Crotaeu (1986), Guenther (1972) and Runeckles and Mabry (1973). The essential oil also is chemically primarily composed of mono – and sesquiterpense and aromatic polypropanoids synthesized via mevalonic pathway for

terpenes and shikmic acid pathway for aromatic poly – propanoids (Runeckles and Mabry, 1973).

Essential also well known to have the range that useful in biological properties against insect, pests, fungal, bacterial and viral diseases (Ibrahim, 2001). In addition, they are more readily degraded in the environment than synthetic compound. Several report also shown that the essential oil from the plant have control on growth of pathogenic strains (Ruberrto et al.,2000 ; Singh et al.,2002 ; Abed, 2007).

The essential oil that mostly in the aromatic plant are the most part volatile and thus, lend themselves to the several technic or method of extraction such as hydro distillation, water and steam distillation, direct steam distillation, and solvent extraction. The specification of the method depends on the plant material that will be distillate to get the desire end-product. Even though some of the less stable complex mixture of organic constituent inside the essential oil may undergo chemical alteration under the steam distillation process, but it is possible that longer the distillation may give more complete oil. Mostly, essential oil is clear, however there are some exceptions. For the example the essential oil of *M.koenigii* having dark yellow color.

2.2.1 Chemical constituent of Essential Oil

An essential oil contains more than 200 chemical components, but some are many times more complex. Essential oils consist of chemical compounds which have hydrogen, carbon and oxygen as their building blocks. They can be essentially classified into two groups:

- Volatile fraction: Essential oil constituting of 90–95% of the oil in weight, containing the monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters.

- Nonvolatile residue: This comprises 1–10% of the oil, containing hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoids.

However the properties of these components can change. For example, the components from the oils extracted from plants can change according to how, when and where these plants are grown and harvested. The constituents can be again subdivided into 2 groups, such as the hydrocarbons which are made up of mostly terpenes and the oxygenated compounds which are mainly alcohols, aldehydes, esters, ketones, phenols and oxides. Some of the common components are listed below along with their properties.

Hydrocarbon:

Building blocks of Essential Oil are hydrogen and carbon. Basic Hydrocarbon found in plants is isoprene having the following structure.

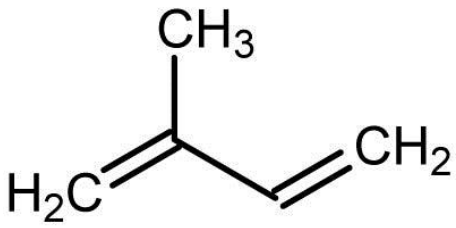

Figure 2.2: Isoprene

- Terpenes:

These components generally have names ending with "ene". Some of them are limonene, pinene, piperene, camphene etc. These components act as an antibacteria l, antiviral, anti-inflammatory, antiseptic, antiviral and bactericidal. These are further categorized intomonoterpene, sesquiterpene and diterpenes. When two of the isoprene units are joined head to tail, the result is a monoterpene, when three are joined, it's a sesquiterpene and similarly four linked isoprene units are diterpenes.

- Monoterpene [$C_{10}H_{16}$]:

Monoterpene are naturally occurring compounds, the majority being unsaturated hydrocarbons (C10).But some of their oxygenated derivatives such as alcohols, Ketones, and carboxylic acids known as monoterpenoids

Two isoprene units are present in these branched-chain C10 hydrocarbons and are widely distributed in nature with more than 400 naturally occurring monoterpenes. Moreover, besides being linear derivatives (Geraniol, Citronellol), the monoterpenes can be cyclic molecules (Menthol – Monocyclic; Camphor – bicyclic; Pinenes (αand β) – Pine genera as well.

Thujone (a monoterpene) is the toxic agent found in Artemisiaabsinthium (wormwood) from which the liqueur absinthe, is made. Borneol and camphor are two common monoterpenes. Borneol, derived from pine oil is used as a disinfectant and deodorant. Camphor is used as a counterirritant, anesthetic, expectorant, and antipruritic, among many other uses.

- Sesquiterpene:

Sesquiterpenes are biogenetically derived from farensyl pyrophosphate and in structure may be linear, monocyclic or bicyclic. They constitute a very large group of secondary metabolites, some having been shown to be stress compounds formed as a result of disease or injury. These are having properties like anti- inflammatory, anti-septic, analgesic and anti-allergic.

- Sesquiterpene Lactones:

These are available as farnesene in chamomile and lavender. They not only have proved to be of interest from chemical and chemotaxonomic point of view, but also possess many antitumor, anti- leukemia, cytotoxic and antimicrobial activities. Chemically the compounds can be classified according to their carboxylic skeletons; thus, guaianolides, pseudoguaianolides, eudesmanolides, eremophilanolides, xanthanolides, etc. can be derived from the germacranolides. Structural features of all these compounds are associated with much of the biological activity. For example beta-caryophyllene in basil and black pepper.

- Diterpenes:

Isoprene has been an integral part in most of the components as there are four isoprene units in diterpenes. By Steam Distillation method we cannot detect diterpenes as this molecule is too heavy to allow for evaporation, so it is rarely found in distilled essential oils. Diterpenes occur in all plant families and consist of compounds having a C20 skeleton.

There are about 2500 known diterpenes that belong to 20 major structural types. Derivatives of diterpenes are plant hormones Gibberellins and phytol occurring as a side chain on chlorophyll. The biosynthesis occurs in plastids and interestingly mixtures of monoterpenes and diterpenes are the major constituents of plant resins. In a similar manner to monoterpenes, diterpenes arise from metabolism of geranyl geranyl pyrophosphate (GGPP). Therapeutically diterpenes have limited importance and are used in certain sedatives (coughs) as well as in antispasmodics and anxiolytics.

- Alcohols:

Naturally Alcohols exist either as a free compound or combined with a terpenes or ester. When terpenes are attached to an oxygen atom, and hydrogen atom, the result is an alcohol. When the terpene is monoterpene, the resulting alcohol is called a monoterpenol. Alcohols are not and are suitable to body or skin. Therefore, they are considered safe to use.Some of these properties are anti-septic, anti-viral, bactericidal and germicidal. Some of the examples are linalool found in ylang-ylang and lavender, geraniol in geranium and rose and nerol in neroli.

- Aldehydes:

Aldehyde containing Essential O ils are effective in treating candida and other fungal infections. Some of these properties are anti- fungal, anti- inflammatory, anti-septic, anti-viral, bactericidal, disinfectant, and sedative. Aldehydes are present as citral in lemon, Citronellal in lemongrass, lemon balm and citrus eucalyptus.

- Acids:

Generally Organic acids are found in very small quantities in their free state within essential oils. Plant acids act as components or buffer systems to control acidity. These also act anti- inflammatory. Examples are cinnamic and benzoic acid in benzoin, citric and lactic.

- Esters:

Esters are formed through the reaction of alcohols with acids. Essential oils containing esters are used for their soothing, balancing effects. Because of the presence of alcohol, they are effective antimicrobial agents. Medicinally, esters are characterized as antifungal and sedative, with a balancing action on the nervous system. They generally are free from precautions with the exception of methyl salicylate found in birch and wintergreen which is toxic within the system. Examples are linlyl acetate in bergamot and lavender and Geranyl formate in geranium.

- Ketones:

Ketones found in plants are used for upper respiratory complaints. They assist the flow of mucus and ease congestion. Essential oils containing ketones are beneficial for promoting wound healing and encouraging the formation of scar tissue. Ketones are (not always) very toxic. The most toxic ketone is Thujone found in mugwort, sage, tansy, thuja and wormwood oils. Other toxic ketones found in essential oils are pulegone in pennyroyal, and pinocamphone in hyssops. Some non-toxic ketones are jasmone in jasmine oil, fenchone in fennel oil, carvone in spearmint and dill oil and menthone in peppermint oil.

- Lactones:

  Lactones are known to be particularly effective for their anti-inflammatory action, possibly by their role in the reduction of prostaglandin synthesis and expectorant actions. Lactones have an even stronger expectorant action than ketones.